Preservation of the Baroreceptor Heart Rate Reflex by Chemical Sympathectomy in Experimental Heart Failure

Luca Mircoli, MD; Luigi Fedele, MD; Marica Benetti, MD; Giovanni Battista Bolla, MD; Alberto Radaelli, MD; Stefano Perlini, MD; Alberto U. Ferrari, MD

Background—The mechanisms underlying impaired baroreflex sensitivity in congestive heart failure (CHF) are incompletely understood. The purpose of the present study was to test the hypothesis that this alteration depends on the marked degree of sympathetic overactivity known to characterize the CHF syndrome.

Methods and Results—Eight-week-old rats were subjected to induction of postmyocardial infarction CHF obtained by coronary ligation (Lig), chronic chemical sympathectomy by 6-hydroxydopamine (Sx), both interventions (Sx-Lig), or neither intervention (Veh-Sham, sham surgery, and vehicle administration). Four weeks after infarction, in conscious state, baroreflex sensitivity was assessed from the bradycardic responses to graded phenylephrine-induced elevations in blood pressure (BP). Left ventricular (LV) diameter was assessed by echocardiography, and plasma catecholamines were assayed to estimate sympathetic activity. Lungs were eventually excised and weighed (LW). CHF was associated with the following: (1) no changes in BP and heart rate; (2) sympathetic overactivity (norepinephrine, 320.2±53.8 pg/mL for Veh-Lig versus 173.4±20.5 pg/mL for Veh-Sham, P<0.01), prevented by Sx (181.2±35.5 pg/mL for Sx-Lig versus 159.8±33.1 pg/mL for Sx-Sham, P=NS); (3) LV enlargement (10.3±0.7 mm for Veh-Lig versus 6.8±0.6 mm for Veh-Sham, P<0.01), irrespective of Sx (9.7±0.7 mm for Sx-Lig versus 6.6±0.5 mm for Sx-Sham, P<0.01); (4) pulmonary congestion (LW, 7.55±0.40 mg per gram of body weight for Veh-Lig versus 5.21±0.44 mg per gram of body weight for Veh-Sham, P<0.01), marginally attenuated by Sx (6.54±0.28 mg per gram of body weight for Sx-Lig versus 4.98±0.22 mg per gram of body weight for Sx-Sham, P<0.05); (5) reduction in baroreflex sensitivity (0.443±0.032 ms/mm Hg for Veh-Lig versus 0.860±0.420 ms/mm Hg for Veh-Sham, P<0.01), entirely prevented by Sx (1.217±0.058 ms/mm Hg for Sx-Lig versus 1.345±0.093 ms/mm Hg for Sx-Sham, P=NS).

Conclusions—In early post-MI CHF, sympathectomy only partially attenuated LV dysfunction and entirely prevented baroreflex sensitivity impairment that arises from enhanced sympathetic activity. (Circulation. 2002;106:866-872.)

Key Words: heart failure ■ baroreceptors ■ nervous system, sympathetic

Congestive heart failure (CHF) has long been known to be associated with an impairment of the arterial baroreceptor reflex.1–5 This is a clinically important phenomenon because it may contribute to altered moment to moment cardiovascular homeostatic adjustments in the CHF patient, and, even more importantly, it has been directly implicated in an enhanced risk of sudden cardiac death6 and total cardiovascular mortality.7 The mechanisms underlying baroreflex dysfunction in heart failure, however, have not been elucidated. Among several other possible contributing factors, one is the marked degree of sympathetic overactivity that is also known to characterize the CHF syndrome.

That sympathetic activity can antagonize baroreflex influences (especially those affecting the heart) was indeed documented by various approaches in both humans and in experimental animals.8–12 In particular, our group showed in recent years that chemical sympathectomy markedly potentiates the baroreceptor heart rate reflex of unanesthetized healthy normotensive rats.13 We thus reasoned that if a normal degree of sympathetic activity physiologically restrains the baroreflex, it may then be that an abnormally high degree of sympathetic activity is responsible for the largely suppressed baroreceptor heart rate reflex associated with CHF.

The purpose of the present study has therefore been to test the hypothesis that chronic chemical sympathectomy prevents the baroreceptor heart rate reflex from being impaired in experimental CHF. Sympathectomy was obtained by 6-hydroxydopamine, heart failure was produced by surgically ligating the left anterior descending coronary artery, thus causing a large anterior myocardial infarction, and the baroreceptor heart rate reflex was evaluated from the bradycardic responses to phenylephrine-induced rises in blood pressure.
Separate groups of ligated and sham-ligated rats subjected to acute or chronic β-adrenoceptor–blocking treatment were also examined.

Methods

Animal Preparation and Surgery
The study was conducted in 36 Sprague-Dawley rats delivered by Charles River Italia (Calco, Italy) at the age of 8 weeks.

Coronary Artery Ligation
Rats were lightly anesthetized with ether and subjected to a left lateral thoracic incision at the 5th intercostal space. The anterior descending coronary branch was ligated by a 6-0 silk thread tightened ∼1 mm distally to the atrioventricular groove. This procedure is followed by development of a large anterior myocardial infarction.14 To minimize the risk of early malignant arrhythmias, lidocaine 1 mg/kg-1 IP was administered immediately after suturing the thoracic wall. Within 4 weeks, the animals reproducibly underwent CHF. A concurrent group of sham animals was subjected to sham coronary ligation: The chest was opened and the thread was passed below the vascular bundle and left in place without being tightened. Coronary ligation procedure was complicated by a significant rate of mortality within 24 hours in rats pretreated with vehicle or propranolol (∼40%) that was even larger among sympathectomized rats (∼60%). No significant changes in mortality rate were observed after the first 24 hours.

Chemical Sympathectomy
Some of the rats were subjected to chemical sympathectomy, as previously described.15 In brief, 4 days before the coronary intervention, 6-hydroxydopamine (100 mg/kg-1) was administered IP; the injections were repeated twice a week. Remaining animals received vehicle injections according to the same schedule. Effectiveness of sympathectomy was verified from the suppression (at least 85% compared with vehicle-treated animals) of the pressor and tachycardic responses to IV tyramine 100 μg/kg-1 performed at the end of the reflex study in the conscious, chronically instrumented rat (see below).

Chronic Propranolol Administration
Separate groups of ligated and sham-ligated rats were subjected to chronic propranolol administration starting 4 days before infarction at the dose of 40 mg/kg-1 per day-1, the drug being dissolved in the drinking water.

Implantation of Intravascular Catheters
Four to five weeks after coronary ligation, each rat was anesthetized by Na pentobarbitone 35 mg/kg-1 IP. Polyethylene catheters were introduced into the femoral artery and vein, tunneled subcutaneously, exteriorized at the interscapular region, and kept patent by periodical flushing with heparinized saline. The animal was then allowed to recover from surgery in an individual cage in which it could move, eat, drink, and explore ad libitum.

Protocol

Echocardiography
Using a 7.5-MHz probe connected to a Sonos HP ultrasound machine, left ventricular (LV) diameter was measured from 2D guided, short-axis parasternal M-mode images. The evaluation was performed during the same anesthesia induced to allow the femoral catheters to be implanted.

Baroreceptor Control of Heart Rate
After at least 24 hours of recovery, the arterial catheter was connected to a Statham P23 D (Oxnard) pressure transducer whose signal was recorded on a Grass 7D chart recorder. Heart rate was obtained beat to beat by tachographic conversion of the pulsatile pressure trace. The catheter transducer system had a flat frequency-response curve up to 30 Hz.

After a 30-minute period of equilibration, bolus injections of phenylephrine were administered in a random sequence at the doses of 0.5, 1, and 2 μg/kg-1 to assess baroreceptor control of heart rate. Each injection was separated from the following one by at least 10 minutes. In 6 ligated and 6 sham-ligated rats, the baroreflex study was repeated after acute administration of propranolol 1 mg/kg-1 IV, the effectiveness of β-adrenoceptor blockade being confirmed by suppression of the tachycardic responses to an intravenous bolus of isoproterenol 0.5 μg/kg-1.

Plasma Catecholamine Assay
On the day after the baroreflex study, 2 mL of arterial blood was drawn in the unanesthetized rat for catecholamine assay (HPLC). Care was taken to minimize behavioral arousal.

All procedures were conducted in accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals (United States Department of Health and Human Services, NIH publication No. 86-23, revised 1992).

Postmortem Assessments
The rat was euthanized by an overdose of pentobarbital, and the heart and lungs were quickly removed and weighed. The presence of an extensive myocardial scar was inspected visually, and infarct size was geometrically assessed as follows: A longitudinal incision along the interventricular groove was performed, and 2 additional transverse incisions were performed to separate the basal, intermediate, and apical portions of the left ventricle. The length and width of the 3 slices was determined under the surgical microscope, and the proportion of infarcted versus healthy tissue was noted. The total infarcted surface was expressed as percentage of the total LV surface to obtain an overall quantitation of the infarct size. Approximately 75% of ligated rats had a large anterior infarction (30% to 40% of total LV surface). Rats with infarcts <30% of the total LV surface were excluded.16

Data Analysis
Baroreflex sensitivity was expressed as the slope of the linear regression relating changes in pulse interval to changes in mean arterial pressure at the peak effect of each phenylephrine injection, provided the correlation coefficient linking the 2 variables was >0.88. Within each experimental group, the resulting slope was calculated and compared by ANOVA to disclose the existence of significant among-group differences; the post hoc Student Newman-Keuls test was then applied to compare baroreflex sensitivities between groups, the level of statistical significance (α index) being set at 0.05.

Other intergroup comparisons included baseline mean arterial pressure, baseline pulse interval, LV end-diastolic diameter, LV fractional shortening, heart to body weight ratio, lung to body weight ratio, plasma norepinephrine, and plasma epinephrine and were performed according to the same statistical method.

Results

LV Diameter and Function
The echocardiographically measured end-diastolic LV diameters were significantly larger in coronary-ligated compared with sham-ligated rats (examples in Figure 1). This difference was similarly observed in the sympathectomized and vehicle-treated groups as well as in the rats with chronic propranolol treatment. LV fractional shortening was markedly and significantly depressed in all ligated compared with sham-ligated rats, with failure of any of the pharmacological treatments to significantly affect this parameter. The complete LV diameter and fractional shortening data are shown in Table 1.
Body and Organ Weights

Body weight was similar in all groups, whereas the normalized weight of the heart was significantly larger in ligated compared with sham-ligated animals (Table 1). The same applied to normalized lung weight, thus documenting the occurrence of substantial pulmonary congestion. The increase in heart and lung weights was observed in the vehicle-treated rats and to a somewhat smaller but still highly significant extent in the chronically propranolol-treated and sympathectomized rats (Table 1).

Baseline Blood Pressure and Pulse Interval and Circulating Catecholamines

As shown in Table 2, there were no significant differences among the various groups with regard to baseline mean arterial pressure, whereas the baseline pulse interval tended to be lower in the ligated, vehicle-treated rats and higher in rats with chronic propranolol treatment.

Plasma norepinephrine was similar in the sham-ligated, vehicle-treated and the sympathectomized rats. In contrast, it was markedly increased in the ligated, vehicle-treated compared with the ligated, sympathectomized rats. In agreement with previous findings, plasma epinephrine was markedly augmented in the sham-ligated, sympathectomized compared with the sham-ligated, vehicle-treated rats (a likely expression of adrenomedullary stimulation in lack of the physiological neural release of catecholamines). In contrast, plasma epinephrine was markedly elevated in both groups of ligated rats. These data are shown in Table 2; no catecholamine assay was performed in the rats chronically treated with propranolol.

Baroreceptor Reflex Sensitivity

Effects of Sympathectomy

Figure 2 shows examples of the changes in arterial pressure and pulse rate in response to phenylephrine injection in one
animal from each of the 4 main experimental groups. It is evident that the reflex bradycardia is virtually completely absent in the ligated, vehicle-treated rat, whereas this is not the case in the ligated, sympathectomized rat in which the reflex response is definitely preserved (if not enhanced) compared with the control, sham-ligated vehicle-treated rat. This pattern was confirmed by the average group data reported in Figure 3, in which the significantly blunted baroreflex response is definitely preserved (if not enhanced) in the ligated, sympathectomized group but the entire normal slopes in the ligated, sympathectomized group are apparent.

**Effects of β-Blockade**

Figure 3 also includes baroreflex findings obtained in the rats with chronic propranolol treatment and shows that such treatment did increase baroreflex sensitivity, although the increase was observed in the ligated but not in the sham-ligated rats, and even in the former group it was quantitatively smaller ($P<0.01$) compared with the effect of chronic sympathectomy. The comparative statistical analysis of the baroreflex sensitivities of ligated and sham-ligated rats with vehicle, chronic propranolol, and chronic 6-hydroxydopamine treatment is summarized in Tables 3 through 5.

In contrast, acute propranolol administration failed to induce any sizable modification of baroreflex sensitivity, which in ligated rats ($n=6$) amounted to 0.465±0.036 (mean±SEM) and 0.492±0.040 ms/mm Hg$^{-1}$ before and after propranolol ($P=NS$); the corresponding values in sham-ligated rats ($n=6$) were 0.897±0.042 and 0.902±0.038 ms/mm Hg$^{-1}$ ($P=NS$).

**Discussion**

The major new finding of our study is that in the conscious rat, chronic sympathectomy prevents the occurrence of the baroreceptor heart rate reflex impairment associated with post-MI CHF. This indicates that the high degree of sympathetic overactivity prevailing in post-MI CHF is a major factor responsible for the impairment of the baroreceptor heart rate reflex typical of this condition. Our conclusions are

### Table 1. End-Diastolic LV Diameter, LV Fractional Shortening, Body Weight, Heart Weight, and Pulmonary Weight in Vehicle-Treated, Sympathectomized, and Chronically Propranolol-Treated Rats Subjected to Either Coronary Ligation or Sham Operation

<table>
<thead>
<tr>
<th></th>
<th>LVD, mm</th>
<th>FS, %</th>
<th>Body Weight, g</th>
<th>Heart Weight, mg/g body weight</th>
<th>Lung Weight, mg/g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEH-SHAM (16)</td>
<td>6.8±0.6</td>
<td>45.2±6.2</td>
<td>380.4±24.2</td>
<td>4.90±0.29</td>
<td>5.21±0.44</td>
</tr>
<tr>
<td>VEH-LIG (17)</td>
<td>10.3±0.7*†‡</td>
<td>18.6±4.6*†‡</td>
<td>403.6±22.7</td>
<td>6.74±0.46*†‡</td>
<td>7.55±0.40*†‡</td>
</tr>
<tr>
<td>SX-SHAM (8)</td>
<td>6.6±0.5</td>
<td>42.6±6.9</td>
<td>376.5±36.7</td>
<td>4.55±0.29</td>
<td>4.98±0.22</td>
</tr>
<tr>
<td>SX-LIG (10)</td>
<td>9.7±0.7*†‡</td>
<td>23.6±3.6*†‡</td>
<td>396.2±26.5</td>
<td>5.78±0.35*†‡§</td>
<td>6.54±0.28*†‡§</td>
</tr>
<tr>
<td>BB-SHAM (8)</td>
<td>6.8±0.6</td>
<td>42.5±5.1</td>
<td>382.5±20.1</td>
<td>4.60±0.30</td>
<td>5.01±0.31</td>
</tr>
<tr>
<td>BB-LIG (8)</td>
<td>9.5±0.7*†‡</td>
<td>20.1±6.0*†‡</td>
<td>386.9±18.5</td>
<td>5.75±0.28*†‡§</td>
<td>6.37±0.26*†‡§</td>
</tr>
</tbody>
</table>

F value 84.36 64.1 2.31 76.04 112.32

* $a<0.01$ vs VEH-SHAM; † $a<0.01$ vs SX-SHAM; ‡ $a<0.01$ vs SX-LIG; § $a<0.05$ vs VEH-LIG.

### Table 2. Mean Arterial Pressure, Pulse Interval, Norepinephrine, and Epinephrine Plasma Levels in the Same Groups as in Table 1

<table>
<thead>
<tr>
<th></th>
<th>MAP, mm Hg</th>
<th>Pulse Interval, ms</th>
<th>Plasma NE, pg/mL</th>
<th>Plasma E, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEH-SHAM (16)</td>
<td>99.6±10.1</td>
<td>161.4±17.5</td>
<td>173.4±20.5 (10)</td>
<td>156.5±40.4 (10)</td>
</tr>
<tr>
<td>VEH-LIG (17)</td>
<td>92.2±10.3</td>
<td>153.6±16.2</td>
<td>320.2±53.8*†‡(11)</td>
<td>403.1±44.8*†‡(11)</td>
</tr>
<tr>
<td>SX-SHAM (8)</td>
<td>95.5±16.7</td>
<td>163.9±14.8</td>
<td>159.8±33.1 (8)</td>
<td>386.1±45.6* (8)</td>
</tr>
<tr>
<td>SX-LIG (10)</td>
<td>92.1±9.8</td>
<td>160.5±15.3</td>
<td>181.2±35.5 (7)</td>
<td>377.4±50.2* (7)</td>
</tr>
<tr>
<td>BB-SHAM (8)</td>
<td>96.3±7.5</td>
<td>178.6±14.9§</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>BB-LIG (8)</td>
<td>92.8±9.2</td>
<td>179.1±15.5§</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

F value 1.06 4.36 3.88 7.26

* $a<0.01$ vs VEH-SHAM; † $a<0.01$ vs SX-SHAM; ‡ $a<0.01$ vs SX-LIG; § $a<0.05$ vs VEH-LIG.
strengthened by two distinct features of our experimental protocol, ie, early implementation of chemical sympathectomy (at the same time as myocardial infarction was produced) and assessment of the status of sympathetic activity by measuring plasma catecholamines (the blood sample being drawn at the time of the baroreflex study). Compared with control animals, vehicle-treated CHF rats did have substantial sympathetic overactivity, whereas such overactivity was abrogated in the 6-hydroxydopamine–treated CHF rats.

An additional finding was that at variance with sympathectomy, chronic β-blockade only partially prevented the heart failure–related impairment of the baroreflex, whereas acute β-blockade had no effect whatsoever.17,18

Although addressing the mechanisms by which sympathetic overactivity can suppress the baroreflex was not part of the study’s objectives, some considerations on this issue are warranted. The agent used to destroy the sympathetic nervous system, 6-hydroxydopamine, is unable to cross the blood-brain barrier; therefore, the sites where the exaggerated sympathetic activity interferes with the baroreflex should be located outside the central nervous system. In lack of any direct experimental evidence on this point, our findings are compatible with the hypothesis that excess peripheral sympathetic drive may have at least 2 targets relevant to the baroreceptor heart rate reflex arch.

- One target is the vagal neuroeffector junction, at which large amounts of released norepinephrine can presynaptically antagonize the release or the action of acetylcholine (which is the preponderant mediator of baroreflex-induced bradycardia).19,20
- The other target is (at the very opposite end of the reflex arch) the wall of the carotid sinus and of the aorta where the baroreceptor organs are located and where arterial distensibility plays a major role in determining mechanoreceptor strain and afferent discharge. We and others have previously demonstrated that sympathetic activity normally exerts a tonic restraint on the distensibility of large arteries, including the carotid artery,21,22 and we have preliminary evidence that in the CHF rat model used in the present study, carotid artery distensibility is markedly reduced.

### BARORECEPTOR-HEART RATE REFLEX

#### TABLE 3. Baroreceptor–Heart Rate Reflex Slopes Observed in the 6 Experimental Groups Shown in Figure 3: Descriptive Statistics of the Regression Slopes

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. Rats</th>
<th>( r (P) )</th>
<th>Slope</th>
<th>SEM Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) VEH-LIG 17 0.89 (0.01) 0.443 0.032</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B) BB-LIG 8 0.92 (0.01) 0.666 0.059</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(C) VEH-SHAM 16 0.94 (0.01) 0.860 0.042</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(D) BB-SHAM 8 0.97 (0.01) 0.982 0.050</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E) SX-LIG 10 0.96 (0.01) 1.217 0.058</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(F) SX-SHAM 8 0.95 (0.01) 1.345 0.093</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( r \) indicates correlation coefficient; \( P \), significance of correlation; and SEM, standard error of the mean. Other abbreviations as in Table 1.
The results of our experiments raise several considerations and hypotheses potentially relevant to the understanding and treatment of clinical heart failure and thus worth being subjected to testing in future studies. First, as mentioned above, an impaired baroreceptor heart rate reflex is associated with and enhanced risk of sudden death in the CHF patient, and it may be that improving the baroreflex (by antiadrenergic drugs as well as by other possible therapeutic tools) will have a favorable prognostic impact. Second, our findings suggest that improvement of the baroreflex may be one of the mechanisms underlying the now well-documented benefit of interfering with the adrenergic nervous system in the CHF patient.23 Third, it is noteworthy that chronic β-blockade and sympathectomy had similar effects on the severity of LV dysfunction and pulmonary congestion, which were slightly attenuated by either treatment, whereas they had distinctly different effects on the baroreflex, which was only partially affected by the former but entirely preserved by the latter treatment. In this regard, an additional question raised by our findings is whether the protective potential of the two interventions may parallel the effect on LV dysfunction and thus be largely superimposable, or may in contrast go “pari passu” with the effect on the baroreflex and thus be much stronger for sympathectomy.

Some possible limitations of our study are also to be mentioned. First, as just mentioned, CHF rats with chronic sympathectomy had a somewhat less severe degree of heart failure compared with their vehicle-treated counterparts, and one might argue that this acts as a confounder in the interpretation of the baroreflex data. Careful review of the results, however, shows that in vehicle-treated CHF rats, baroreflex sensitivity was reduced by 49% (P<0.01), whereas LV diameter, LV fractional shortening, normalized heart weight, and normalized lung weight were increased by 47%, 37%, and 45%, respectively, compared with their non-CHF counterparts (vehicle-treated, sham-ligated). The corresponding values for sympathectomized CHF rats compared with their non-CHF counterparts were −10% (P=NS), +46%, +27%, and +31%. In a similar way, LV fractional shortening was reduced in rats with CHF (−55% in vehicle-treated ligated rats versus vehicle-treated sham-ligated) irrespective of sympathectomy (−45% in sympathectomized ligated rats versus sympathectomized sham-ligated). Thus, the difference in baroreflex sensitivity between the 2 groups was substantial, whereas the difference in the severity of CHF was not, and it should be highly unlikely that the minor difference in the severity of CHF may have accounted for the observed changes in baroreflex sensitivity to any relevant extent. Furthermore, chronic propranolol treatment attenuated heart failure severity to the same extent as sympathectomy but failed to similarly preserve the baroreflex, indicating that such attenuation is not a strict determinant of the baroreflex improvement.

A second aspect to be considered is that our rats were studied at a relatively early stage of CHF (4 weeks after MI), so that our major conclusion that sympathectomy prevents the baroreflex from being impaired in the course of heart failure is accordingly to be restricted to this early stage, and it cannot be excluded that in association with a longstanding heart failure, the preventive action of sympathectomy is partially or totally lost.

In conclusion, we demonstrated that in a rat model of early post-MI heart failure, the impairment of the baroreceptor heart rate reflex depends on sympathetic overactivity and can be prevented by chronic sympathectomy, which implies that the phenomenon originates from a functional derangement rather than from putative structural defects of the cardiovascular system. Furthermore, considering the prognostic relevance of baroreflex dysfunction in heart failure, our data strengthen and refine the concept that attenuating sympathetic overactivity may be an important therapeutic target in this syndrome.

References


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